

AMENDMENTS TO THE SPECIFICATION

Immediately after the Title of the Invention please add the following paragraph:

This application is U.S. National Phase of International Application PCT/NZ2004/000333, filed December 22, 2004 designating the U.S., and published in English as WO 2005/061699 on July 7, 2005, which claims priority to New Zealand Patent Application No. 530331, filed December 22, 2003.

On page 22 of the Specification please replace the following paragraphs under the header “BRIEF DESCRIPTION OF DRAWINGS”:

Figure 4. The nucleotide sequence of *N. lolii* strain Lp19 *ltmG* (SEQ ID NO: 1).

Figure 5. The polypeptide sequence of *N. lolii* strain Lp19 *LtmG* (SEQ ID NO: 2).

Figure 6. The nucleotide sequence of *N. lolii* strain Lp19 *ltmM* (SEQ ID NO: 3).

Figure 7. The polypeptide sequence of *N. lolii* strain Lp19 *LtmM* (SEQ ID NO: 4).

Figure 8. The nucleotide sequence of *N. lolii* strain Lp19 *ltmK* (SEQ ID NO: 5).

Figure 9. The polypeptide sequence of *N. lolii* strain Lp19 *LtmK* (SEQ ID NO: 6).

Figure 10. The nucleotide sequence of *N. lolii* strain Lp19 *ltmG*, *ltmM* and *ltmK* gene cluster (SEQ ID NO: 23).

Figure 11. The nucleotide sequence of *E. festucae* strain F11 *ltmG* (SEQ ID NO: 17).

Figure 12. The nucleotide sequence of *E. festucae* strain F11 *ltmM* (SEQ ID NO: 19).

Figure 13. The nucleotide sequence of *E. festucae* strain F11 *ltmK* (SEQ ID NO: 21).

Figure 14. The polypeptide sequence of *E. festucae* strain F11 *LtmG* (SEQ ID NO: 18).

Figure 15. The polypeptide sequence of *E. festucae* strain F11 *LtmM* (SEQ ID NO: 20).

On page 23 of the Specification please replace the following paragraphs under the header “BRIEF DESCRIPTION OF DRAWINGS”:

Figure 16. The polypeptide sequence of *E. festucae* strain F11 *LtmK* (SEQ ID NO: 22).

Figure 19. An EST derived nucleic acid fragment from the suppressive subtractive hybridization library with homology to *Penicillium paxili* paxP (SEQ ID NO: 52).

Figure 20. An EST derived nucleic acid fragment from the suppressive subtractive hybridization library with homology to *Penicillium paxili* paxP (SEQ ID NO: 53).

Figure 21. An EST derived nucleic acid fragment from the suppressive subtractive hybridization library with homology to *Penicillium paxili* paxP (SEQ ID NO: 54).

Figure 22. An EST derived nucleic acid fragment from the suppressive subtractive hybridization library with homology to *Penicillium paxili* paxD (SEQ ID NO: 55).

Figure 23. An EST derived nucleic acid fragment from the suppressive subtractive hybridization library with homology to *Penicillium paxili* paxD (SEQ ID NO: 56).

Figure 24. An EST derived nucleic acid fragment from the suppressive subtractive hybridization library with homology to *Penicillium paxili* paxD (SEQ ID NO: 57).

Figure 25. An EST derived nucleic acid fragment from the ~~an~~ *in vitro* culture library with homology to cytochrome P450 monooxygenases (SEQ ID NO: 58).

On page 24 of the Specification please replace the following paragraphs under the header “BRIEF DESCRIPTION OF DRAWINGS”:

Figure 28. The nucleotide sequence of *N. lolii* strain Lp19, cluster 2, *ltmP*-rev, *ltmQ*, *ltmD*, *ltmC*-rev, *ltm25* (SEQ ID NO: 24).

Figure 29. The nucleotide sequence of *N. lolii* strain Lp19 *ltmC* (SEQ ID NO: 7).

Figure 30. The polypeptide sequence of *N. lolii* strain Lp19 *ltmC* (SEQ ID NO: 8).

Figure 31. The nucleotide sequence of *N. lolii* strain Lp19 *ltmP* (SEQ ID NO: 9).

Figure 32. The polypeptide sequence of *N. lolii* strain Lp19 *ltmP* (SEQ ID NO: 10).

Figure 33. The nucleotide sequence of *N. lolii* strain Lp19 *ltmQ* (SEQ ID NO: 13).

Figure 34. The polypeptide sequence of *N. lolii* strain Lp19 *ltmQ* (SEQ ID NO: 14).

Figure 35. The nucleotide sequence of *N. lolii* strain Lp19 *ltm25* (SEQ ID NO: 59).

Figure 36. The polypeptide sequence of *N. lolii* strain Lp19 *ltm25* (SEQ ID NO: 60).

On page 25 of the Specification please replace the following paragraphs under the header “BRIEF DESCRIPTION OF DRAWINGS”:

Figure 37. The nucleotide sequence of *N. lolii* strain Lp19 *ltmD* (SEQ ID NO: 15).

Figure 38. The polypeptide sequence of *N. lolii* strain Lp19 *ltmD* (SEQ ID NO: 16).

Figure 40. The nucleotide sequence of *N. lolii* strain Lp19, *ltm* cluster 3, *ltmE* and *ltmJ* (SEQ ID NO: 25).

Figure 41. The nucleotide sequence of *N. lolii* strain Lp19 *ltmJ* (SEQ ID NO: 11).

Figure 42. The polypeptide sequence of *N. lolii* strain Lp19 *ltmJ* (SEQ ID NO: 12).

Figure 43. The nucleotide sequence of *N. lolii* strain Lp19 *ltmE* (SEQ ID NO: 61).

Figure 44. The polypeptide sequence of *N. lolii* strain Lp19 *ltmE* (SEQ ID NO: 62).

On page 28 of the Specification please replace the following Table:

TABLE 2: Primer List

Name	sequence 5'→3'	amplifies	SEQ ID NO
CY 4	GCT TGG ATC CGA TAT TGA AGG AGC	hph/BamHI	<u>29</u>
CY 5	TTG GAT CCG GTT CCC GGT CGG CAT	hph/BamHI	<u>30</u>
ggpps 27	CAY MGI GGT CAR GGT ATG GA	dPCR	<u>26</u>
ggpps 28	TTC ATR TAG TCG TCI CKT ATY TG	dPCR	<u>27</u>
ggpps 29	AAC TTT CCY TCI GTS ARG TCY TC	dPCR	<u>28</u>
lol 1	TGG ATC ATT CGC AGA TAC	<i>ltmG</i>	<u>31</u>
lol 2	GTG TGA GAT TAA GAC GTC	LHS	<u>32</u>
lol 3	ACC GAC GCC ATT AAT GAG	<i>ltmG</i>	<u>33</u>
lol 7	ACT GGG CAT CTT CCA TAG	<i>ltmM</i> /mid	<u>34</u>
lol 14	ATT AGA GGC ACC GAA CGC	RT- PCR <i>ltmM</i>	<u>35</u>
lol 15	ATC AAG CTG GCT ATC CTC	<i>ltmP</i>	<u>32</u>
lol 17	AAA TAA TGG GCA AGG AGC	KO PstI	<u>37</u>
lol 18	TGG GAAT TTT GGA AAT GGC	KO PstI	<u>38</u>
lol 28	GCT CCT TGC CCA TTA TTT	RT-PCR <i>ltmM</i>	<u>39</u>
lol 29	GTC TTG ATC GTC TGC ATC	RT-PCR <i>ltmP</i>	<u>40</u>
lol 32	TGT CCG TGC ATC CAT TGT	<i>ltmP</i>	<u>41</u>
lol 34	CAT AGA GCT AGC TAG AGT	LHS	<u>42</u>
lol 35	GTT CGG TGC CTC TAA TAC	<i>ltmM</i> /mid	<u>43</u>
lol 43	GAG GAT AGC CAG CTT GAT	RT-PCR <i>ltmP</i>	<u>44</u>
lol 48	GAT TGG TAC CTT GAA GTC GCT AGT	KO KpnI	<u>45</u>
lol 49	GTA GGG TAC CTC TAG TAC TGC CTC T	KO KpnI	<u>46</u>
lol 63	TAG CGA ATC ATT GCG TCG	RT-PCR <i>ltmP</i>	<u>47</u>
lol 79	ATG GCT GCC AAT GAC TTT CC	RT-PCR <i>ltmG</i>	<u>48</u>

lol 135	AGG CCA TTT TCG ACA GTT GT	KO integration	<u>49</u>
lol 147	CCA GCA AGC ATG CAC ATT AC	RHS	<u>50</u>
lol 148	TGC GTG AGA GAT AAA GCA AG	KO integration	<u>51</u>
pUC forward	GCC AGG GTT TTC CCA GTC ACG A		<u>63</u>
pUChph 3	CTG CAT CAT CGA AAT TGC	hph	<u>64</u>
pUChph 4	AAA CCG AAC TGC CCG CTG TTC	hph	<u>65</u>
PUC reverse	GAG CGG ATA ACA ATT TCA CAC AGG		<u>66</u>
T7	TAA TAC GAC TCA CTA TAG GG		<u>67</u>

On page 29 of the Specification please replace the following paragraph:

Sequence data was assembled into contigs using SEQUENCHER version 4.1 (Gene Codes) and analyzed using the Wisconsin Package version 9.1 (Genetics Computer Group, Madison, Wisconsin). Sequence comparisons were performed through Internet Explorer version 6.0 at the National Center for Biotechnology Information (NCBI) site (<http://www.ncbi.nlm.nih.gov/>) using the Brookhaven (PDB), SWISSPROT and GenBank (CDS translation), PIR and PRF databases employing algorithms for both local (BLASTX and BLASTP) and global (FASTA) alignments (Pearson and Lipman 1988; Altschul et al. 1990; Altschul et al.1997).

On page 41 of the Specification please replace the following paragraph under the header “Template preparation and Library sequencing”:

For sequencing template preparation PCR reactions were carried out in 384-well plates using the M13 forward (GTAAAACGACGGCCAG) (SEQ ID NO: 68) and Reverse primers (CAGGAAACAGCTATGAC) (SEQ ID NO: 69). The Biomek 2000 liquid handling robot was used to transfer 1 µl aliquots from each of 4 x 96-well plates containing overnight cultures into a conical bottomed 384-well plate (ABGen). PCR products were precipitated using 1 µl of 3M NaOAC (pH 6) and 15 µl of isopropanol and placed at -80°C for at least one hour before centrifugation at 4K for 1 hr (4°C). Pellets were washed with 20 µl of 70% ethanol and

centrifuged for a further 30 min at 4K (4°C) before they were air dried and resuspended in 10 µl of sterile MQ water. Products were checked by running 1 µl samples on a 1% agarose gel (1X TAE).

On page 51 of the Specification please replace the following paragraph under the header “PCR analysis”:

Individual colonies from converted libraries were inoculated into 100 µl of LB broth containing carbenicillin (50 µg/ml) in round bottomed 96-weil plates (Nunc). Plates were incubated overnight at 37°C. Aliquots of 1 µl of each overnight culture were PCR amplified in a total volume of 15 µl using ptriplex2FORWARD (5'- AAGCGCGCCATTGTGTTGGTACCC-3') (SEQ ID NO: 70) and ptriplex2REVERSE (5'- CGGCCGCATGCATAAGCTTGCTCG-3') (SEQ ID NO: 71) as primers (present in the pTriplEx vector arms) (Kohler et al., 2003). The PCR included 95°C for 3 min, 95°C for 60 s, 60°C for 30 s, 72°C for 3 min for 30 cycles and a final extension of 72°C for 15 min (iCycler, Bio-Rad, USA). One µl of each reaction was analysed on a 1% agarose gel alongside 0.25 µg of a 1 kb plus DNA standard (Invitrogen) and stained with ethidium bromide to determine the size and quality of the PCR products.

On page 28 of the Specification please replace the following Table:

Table 9 Primers Used in this example and not listed in table 2

Primer name	Sequence 5' - 3'	Used for	SEQ ID NO
lol191	CCAAAGGAGGTTTTGAATGTA	<i>ltmP</i> PCR/probe	<u>72</u>
lol192	TTGGATGAGCTCAATCATGC	<i>ltmP</i> PCR/probe/RT-PCR	<u>73</u>
lol194	GAAGTCGTAGCGCAGGAGCA	<i>ltmJ</i> PCR	<u>74</u>
lol195	TTCTCTTCGGAGGCTCTCCT	<i>ltmP</i> PCR	<u>75</u>
lol196	TGGACATGGATCTGATTGTC	<i>ltmP</i> probe	<u>76</u>
lol198	TGTAGCACGGGTAGCTAGAT	<i>ltmP</i> probe	<u>77</u>
lol199	TTGCGCATCGTACGCTAGGA	IPCR	<u>78</u>
lol202	GGATGAAGAAAATCCACGAG	IPCR	<u>79</u>
lol203	AGACGATCTGTTAGGCCGAT	IPCR	<u>80</u>

lol205	CCAAGCATCGATTTGTCACC	<i>ltmJ</i> PCR/probe	<u>81</u>
lol206	AATCTGATCGCCATCTTTGC	<i>ltmJ</i> PCR/probe	<u>82</u>
lol209	GAATAGCTCAAGACTCAGAA	IPCR	<u>83</u>
lol210	AAGCTGGCTGTAAAGGGTC	IPCR	<u>84</u>
lol211	TATTAGGGAGCGAACTTCAC	IPCR	<u>85</u>
lol213	AAGAGGGCCGCAATTTCGAT	IPCR	<u>86</u>
lol222	GCGTGCAACATTAACATTCTC	IPCR	<u>87</u>
lol235	ATTCCACCATGGCATCTGGAGCATGGCTC G	<i>ltmC</i> complementation complenetation	<u>88</u>
lol236	CTTAAGCGAATTCTACCTTGTGGGTC	<i>ltmC</i> probe/complementation	<u>89</u>
lol341	TTCCGCTTCCGAGTAGACTC	<i>ltmE</i> PCR/RT- PCR/probe	<u>90</u>
lol356	CCGAGTTTGATGACCTGCTG	<i>ltmE</i> PCR/RT- PCR/probe	<u>91</u>
SP6	CCATTTAGGTGACACTATAG	Seq	<u>92</u>
Tl.1	GAGAAAATGCGTGAGATTGT	<i>Tub2</i> probe/RT-PCR	<u>93</u>
Tl.2	CTGGTCAACCAGCTCAGCAC	<i>Tub2</i> probe/RT-PCR	<u>94</u>